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14. ABSTRACT
Thus far we have recruited 14 breast cancer (BCa) patients. The women were stratified according to their vitamin D status: 9 have normal circulating levels of vitamin D (>30 ng/mL), 3 have insufficient levels of vitamin D (17-29 ng/mL) and 2 have deficient levels (<16 ng/mL). We have intervened in the neo-adjuvant period between biopsy and surgery and treated the insufficient patients with 2000 IU of vitamin D and the deficient patients with 4000 IU of vitamin D. We will analyze their biopsy samples (pre-intervention) and compare results with the surgical specimens (post-intervention) to determine whether dietary vitamin D supplements can regulate the gene expression profile in the BCa. Further, we will determine whether vitamin D deficiency is associated with a poor prognosis profile and whether vitamin D intervention converts the profile toward a more normal profile of gene expression, perhaps representing a better prognosis.

15. SUBJECT TERMS
vitamin D, calcitriol, breast cancer, vitamin D receptor, estrogen receptor, antiproliferation, COX-2, prostaglandins, aromatase

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Introduction

Although the grant was supposed to start 9/1/07 the DOD human subjects approval process greatly delayed the start date. We finally received human subjects approval in mid-July and our Notice of Award was received on 3/13/08. We actually initiated work on the grant and began to charge effort to the grant on 4/1/08. Therefore this report contains our efforts for the time from 4/1/08 to 8/31/08, a period of only 5 months. It is our intention to work on this project for 2 full years and to request a no cost extension on 8/31/09 to continue funding these efforts until the 2 years are completed.

The goals of the study are unchanged from the original grant. The recruitment of patients is underway and the findings will be detailed below.

The work to be accomplished as described in the SOW.

1. To recruit patients undergoing core needle biopsy for breast abnormalities so as to accrue 50 evaluable patients with breast cancer to study.
2. To analyze their vitamin D, PTH, and calcium status
3. To characterize the classical prognostic and predictive characteristics of the breast cancer at time of diagnostic biopsy
4. To analyze the cancer biopsy specimens for a gene profile
5. To determine whether the prognostic and predictive factors differ between vitamin D deficient or insufficient patients and vitamin D sufficient patients.
6. To treat the vitamin D deficient and insufficient patients with vitamin D during the interval between biopsy and definitive breast surgery
7. To reanalyze the vitamin D status after intervention and before surgery
8. To analyze the surgical cancer specimens for their gene profile and compare the findings to the core biopsy gene profile to determine whether vitamin D therapy changes the profile
9. To compare the results of treated deficient/insufficient patients to sufficient patients to determine whether vitamin D intervention normalized the gene profile.
10. To analyze the data

Body

Recruitment of subjects with breast cancer is going well. At this time we have consented 14 patients. Their vitamin D [25(OH)D] levels are shown in the accompanying table. The patients have been stratified to receive vitamin D therapy depending on their initial 25(OH)D levels. 9 had normal vitamin D, 3 had insufficient levels and 2 had deficient levels. The insufficient patients were treated with 2000 IU of vitamin D and the deficient patients with 4000 IU of vitamin D. Of the total number of subjects 11 have undergone surgery for their breast cancer.

At this time we have not yet started to analyze the gene profile of the subjects and the biopsy samples are being stored. We have decided that it would be most efficient and provide the most reliable data if we were to analyze both the biopsy and surgical samples of each patient in the same analysis. Also, for better comparison, we plan to analyze several patients at the same time. Therefore the samples are awaiting analysis. At this time we are optimizing the strategy to assess the gene expression profile of the subjects.

A potential problem that has surfaced is the extremely small amount of tissue left for our study after the pathologist has completed the clinically relevant analysis of the biopsy material. We have found that the yield of RNA from this residual amount of tissue is not sufficient to perform individual PCR analyses on the full complement of genes that we planned to study. We are therefore investigating the technique of RNA amplification to generate enough RNA for the full analysis. If we do this successfully, it will also allow us to do

full cDNA microarrays on selected patients. Alternatively, newer techniques that allow multiplexing of PCR analyses have become available since the grant was written. These techniques will allow PCR analysis of many gnes from a small amount of RNA. We are working to establish the best methods to do the gene expression profile during this period of patient recruitment.

Key Research Accomplishments

Recruitment of 14 subjects to the study.

Reportable Outcomes

None yet

Conclusions

The recruitment of patients is going well. There are fewer vitamin D deficient patients than we expected based on published ratios of vitamin D levels in the population. However we are finding women with breast cancer that have a distribution of vitamin D levels so that the three strata (no vitamin D, 2000 IU vitamin D, and 4000 IU vitamin D) are already represented in our study. We anticipate that patient recruitment will be successful. In the mean time, we are optimizing the strategy to evaluate the profile of gene expression in the breast cancer biopsies and surgical specimens.

References

N/A

Appendices

Table 1 showing data on recruited subjects including vitamin D levels and intervention.

Table 1. Data describing recruited patients for breast cancer trial.

ID	Age	Race/ Ethnicity	Consent date	Breast Cancer Status	ER status	PR status	Vitamin D 25		Vitamin D 1,25		Calcium		PTH		Type of Surgery	Surgery Status	Vitamin D intervention
							Baseline	Pre-surgery	Baseline	Pre-surgery	Baseline	Pre-surgery	Baseline	Pre-surgery			
1	49	White/Non Hispanic	4/28/2008	Invasive Lobular Carcinoma	Positive	Positive	45	45	23	36	8.7	8.8	27	21	Mastectomy	Completed	No Intervention
2	53	Asian/Non Hispanic	4/28/2008	Invasive Ductual Carcinoma	Negative	Negative	35	36	38	44	9.5	9.2	27	51	Lumpectomy	Completed	No Intervention
3	45	White/Non Hispanic	4/29/2008	Invasive Ductual Carcinoma	Positive	Positive	26	36	75	62	9.2	9.1	30	31	Mastectomy	Completed	One Capsule for 37 days
4	47	Other/Hispanic	5/9/2008	Invasive Lobular Carcinoma	Awaiting results	Awaiting results	50	Not Done	58	Not Done	9.5	Not Done	11	Not Done	Mastectomy	Completed	No Intervention
5	46	Asian/Non Hispanic	5/20/2008	Invasive Mixed ductal and micropapillary	Positive	Positive	14	26	36	57	9.5	9.5	31	33	Mastectomy	Completed	Two Capsules for 12 days
6	35	White/Non Hispanic	6/5/2008	Invasive Ductual Carcinoma	Negative	Negative	33	35	98	45	9.7	9.5	5	21	Mastectomy	Completed	No Intervention
7	50	White/Non Hispanic	6/27/2008	Invasive Ductual Carcinoma	Positive	Positive	19	37	41	62	8.5	8.7	82	59	Lumpectomy	Completed	One Capsule for 49 days
8	47	Asian/Non Hispanic	6/26/2008	Invasive Ductual Carcinoma	Positive	Positive	27	35	45	48	9.1	9.6	20	18	Mastectomy	Completed	One Capsule for 14 days
9	50	White/Non Hispanic	7/17/2008	Invasive Ductual and Tubular Carcinoma	Positive	Positive	39	41	63	71	9.8	8.9	25	29	Lumpectomy	Completed	No Intervention
10	37	White/Non Hispanic	7/17/2008	Invasive Ductual Carcinoma	Awaiting results	Awaiting results	38	26	60	56	9.7	7.9	36	25	Mastectomy	Completed	No Intervention
11	57	White/Non Hispanic	7/29/2007	Invasive Ductual Carcinoma	Awaiting results	Awaiting results	32	9/2/2008	47	9/2/2008	10	9/2/2008	76	9/2/2008	Lumpectomy	9/3/2008	No Intervention
12	71	White/Non Hispanic	7/30/2008	Invasive Mucinous Carcinoma	Awaiting results	Awaiting results	11	20	27	31	9.5	9.0	73	78	Lumpectomy	Completed	Two Capsules for 15 days
13	54	White/Non Hispanic	8/7/2008	Invasive Ductual Carcinoma	Awaiting results	Awaiting results	49	9/25/2008	62	9/25/2008	9.1	9/25/2008	31	9/25/2008	Not decided	9/25/2008	No Intervention
14	56	White/Non Hispanic	8/26/2008	Invasive Ductual Carcinoma	Positive	Positive	51	Surgery not Scheduled	Awaiting results	Surgery not Scheduled	9.4	Surgery not Scheduled	42	Surgery not Scheduled	Not decided	Surgery not Scheduled	No Intervention